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The total glycoalkaloid (TGA) contents and individual glycoalkaloid compositions of foliage from 16 wild, tuber-bearing *Solanum* species of potential use in potato breeding were determined by using a combination of analytical procedures. A wide range of TGA contents was found (0.2–39.0 mg/g dry weight). Most of the species had higher TGA levels than *Solanum tuberosum*, cv. Katahdin (2.6 mg/g dry weight). Great diversity in individual glycoalkaloid composition was found among the species. Most species contained only α-solanine and α-chaconine, but glycoalkaloids not usually associated with commercial cultivars were found in several species. Glycoalkaloids (probably solamargine and solasonine) not previously found in any tuber-bearing *Solanum* species were found in *Solanum berthaultii* (PI 265858). This report also provides glycoalkaloid data on several previously unanalyzed species. The data emphasize the need for careful evaluation of glycoalkaloids as a potato quality factor in breeding programs which exploit wild *Solanum* species.

The total glycoalkaloid (TGA) levels and individual glycoalkaloid compositions of current potato cultivars do not represent a toxicological or teratogenic hazard. However, the widespread exploitation of wild, tuber-bearing *Solanum* species in potato breeding programs suggests the need for caution in the future. Some species contain much higher TGA levels than are found in *Solanum tuberosum* (Schreiber, 1963; Osman et al., 1978). There is also widespread occurrence among these wild species of glycoalkaloids which are not commonly associated with commercial varieties [for a review, see Schreiber (1968)]. Since TGA levels and individual glycoalkaloid compositions are largely under genetic control (Sanford and Sinden, 1972; McCollum and Sinden, 1979), and glycoalkaloids have been associated with undesirable potato flavor (Sinden and Deahl, 1976), mammalian toxicity and teratogenicity [for a review, see Kuĉ (1975)], careful consideration of glycoalkaloid contents in the parental species is highly desirable. The need for this was illustrated in the late 1960s when a Gr. Tuberosum cultivar Lenape, which had *S. chacoense* in its ancestry (Akeley et al., 1968), was withdrawn from commerce because of its high TGA content (Zitnak and Johnston, 1970; Burton, 1974). This problem might have been avoided if the TGA content of *S. chacoense*, which is very high (Schreiber, 1963; Tingey et al., 1978), had been considered. Most of the current information on the glycoalkaloid contents of wild potato species was published prior to 1965. Since then, improved methodology has facilitated more sensitive, accurate analyses which have already led to new discoveries. Whereas commercial varieties of *S. tuberosum* were thought to contain only the solanidine glycoalkaloids α-chaconine and α-solanine, Shih and Kuĉ (1974) demonstrated the presence of the tomatidenol glycoalkaloids α- and β-solamarine in leaves and incubated slices of the cultivar Kennebec. In addition, Osman et al. (1976) reported the presence of a new glycoalkaloid, commersonine, in certain lines of *S. chacoense* and *S. commersonii*.

We have analyzed 16 wild, tuber-bearing *Solanum* species for TGA and individual glycoalkaloid composition, using a combination of highly sensitive, accurate techniques. This report provides an update on previous literature, some of which may be misleading, and also presents glycoalkaloid data on several species of potential use in breeding which were previously unanalyzed.

MATERIALS AND METHODS

Germ Plasm. The wild, tuber-bearing *Solanum* accessions were obtained from the Potato Introduction Station, Sturgeon Bay, WI. The materials were grown at Freeville, NY, and treated in the same manner as previously described (Raman et al., 1979).

Total Glycoalkaloid Content. Freeze-dried leaf powders (0.2–1 g) were homogenized in 5% acetic acid (~40 mL/g of powder) with a Polytron homogenizer operating at full speed for 90 s. Extracts were filtered and the filter was washed twice with 5-mL aliquots of the extraction medium. For determination of the contents of ammonia-precipitable glycoalkaloids in extracts, aliquots (100–500 mg of powder) were precipitated by adding

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Table I. Foliar Glycoalkaloid Contents of Some Wild, Tuber-Bearing *Solanum* Accessions

species	accession	total glycoalkaloid concn mg/g dry wt[f] ammonia ppt	non-ppt	total	glycoalkaloids, % of total α-chaconine	α-solanine	other[e]
acaule	PI 225620	5.0	2.8[g]	7.8	*a*	*a*	100,[b] demissine
berthaultii	PI 265858	1.0	0.0	1.0	*a*	*a*	unidentified[d]
bulbocastanum	WRF 1565	0.2	0.0	0.2	*a*	*a*	unidentified
brachycarpum	PI 275179	35.0	4.0	39.0	24	17	37, β-solamarine; 22, α-solamarine
chacoense	PI 189220	29.0	0.0	29.0	65	35	*a*
chacoense	PI 320285	14.0	15.0[g]	29.0	15	15	57, leptinines I and II; 13, leptines I and II
chacoense	WRF 888	27.0	4.0	31.0	53	36	7, leptinines I and II; 4, leptines I and II
commersonii	PI 320269	23.0	0.0	23.0	*a*	*a*	100,[b] dehydrocommersonine
fendleri	PI 225543	3.5	1.0	4.5	88	12	*a*
hjertingii	PI 257067	18.0	2.0	20.0	51	49	*a*
hougasii	PI 161174	29.0	3.0	32.0	75	25	*a*
hougasii	PI 161726	26.0	3.0	29.0	63	37	*a*
kurtzianum	WRF 335	17.0	2.0	19.0	85	15	*a*
medians	PI 283081	6.6	1.1	7.7	57	43	*a*
pinnatisectum	PI 186554	18.0	3.0	21.0	*a*	*a*	100,[b] tomatine
polyadenium	PI 161728	8.7	11.3[g]	20.0	*a*	*a*	100, tomatine
polytrichon	PI 184773	2.5	*c*	2.5	61	39	*a, c*
sucrense	PI 230465	8.3	*c*	8.3	57	43	*a, c*
tuberosum	Katahdin	2.6	*c*	2.6	73	27	*a, c*

[a] None detected or levels too low (<3%) to identify. [b] No other glycoalkaloids or aglycons detected or levels too low (less than ~3%) to identify; greater than 95% of aglycon peak areas in identified glycoalkaloids. [c] TGA concentration estimated by titration of ammoniacal precipitates only. Aqueous extract not hydrolyzed. [d] Solasodine aglycon was identified [TLC (Hunter et al., 1976), GLC, GLC–MS, MP, and mixed MP]. Composition of sugar moieties was determined by GLC (Varma et al., 1973). Probably solamargine and solasonine. [e] Identified by GLC and TLC of glycoalkaloids (cochromatographed), GLC of aglycons, and TLC of aglycons (cochromatographed). [f] Concentration calculated by adjusting titration value (tomatine standard) for the molecular weight of glycoalkaloids known to be present. [g] A portion of the total glycoalkaloid(s) in certain extracts was not precipitated and pelleted by the ammoniacal precipitation procedure used. Presence of the specified quantities of glycoalkaloid(s) in the ammoniacal supernatants of these three extracts (*S. acaule*; *S. chacoense* PI 320285; *S. polyadenium*) was also established by TLC of concentrated supernatant and GLC of aglycons liberated by hydrolysis of concentrated supernatants with 1 N methanolic HCl.

enough concentrated ammonia to raise the pH to 11.0. The ammoniacal extract was then warmed to 70 °C and cooled overnight at 4 °C, and the precipitate was collected by centrifugation (Bushway et al., 1980). After the pellet was washed twice with 1% ammonia and dried (16 h at 70 °C in a vacuum oven), the glycoalkaloids were dissolved in an appropriate volume of methanol, filtered, and, following acid hydrolysis, titrated with bromophenol blue in 10% phenol (Fitzpatrick and Osman, 1974).

For determination of the contents of ammonia-soluble glycoalkaloids in *S. chacoense* foliage (Kuhn and Löw, 1961) and in foliage from other species that also might have ammonia-soluble glycoalkaloids, aliquots (50–400 mg of powder) of the acetic acid extracts were evaporated to dryness under an air stream in a boiling water bath and hydrolyzed. For hydrolysis, glycoalkaloids were dissolved in 2 mL of 1 N methanolic HCl and hydrolyzed for 6 h at 68 °C in a sealed, screw-capped test tube. The liberated aglycons were extracted from the hydrolysate by adding 4 mL of concentrated ammonia to the cooled tube, followed by 2 mL of dichloromethane. After vigorous mixing (Vari-Whirl) and phase separation, the dichloromethane (lower) layer was removed with a Pasteur pipet. This extraction procedure was repeated with two additional aliquots of dichloromethane, and the combined dichloromethane extracts were washed twice with water. The aglycon extract was then evaporated to dryness under an air stream.

The quantity of aglycons was determined by titrating samples of the aglycon extracts (Fitzpatrick and Osman, 1974). The differences found between the titer of the ammonia-precipitated sample and the titer of the aglycon extract from the hydrolysate of the nonprecipitated extract of the sample was used to estimate the quantity of nonprecipitable glycoalkaloids present in an extract. In certain extracts, i.e., *S. acaule*, *S. chacoense* PI 320285, and *S. polyadenium*, a considerable portion of the total glycoalkaloid was found in the supernatant of the ammoniacal precipitate. So that the validity of the method of estimating ammonia-soluble and/or nonprecipitable glycoalkaloid quantities could be tested by determining the difference between the total TGA and the portion of the total TGA found by titrating the ammoniacal precipitate, supernatant fractions from these three species were analyzed directly. The ammoniacal supernatant was reduced to dryness under an air stream in a boiling water bath, 5 mL of water was added, and the drying process was repeated. Glycoalkaloids were dissolved in methanol and filtered, and an appropriate aliquot analyzed by TLC for glycoalkaloid composition. An aliquot was hydrolyzed and analyzed by GLC for aglycon composition. Concentrations of aglycons were estimated by comparing peak area(s) on GLC chromatograms with peak area(s) on chromatograms of standards. The differences found between the concentrations of nonprecipitable glycoalkaloids by using the two methods of estimating nonprecipitated glycoalkaloids in these extracts were less than the differences caused by experimental error when replicated analyses of a few selected leaf powders were conducted.

Individual Glycoalkaloid Composition. Samples of the nonprecipitated extracts were permethylated, and the permethylated derivatives separated by GLC (Herb et al., 1975) in order to estimate the relative amounts of each glycoalkaloid. In addition, samples of the aglycon extracts were analyzed for quantity and types of aglycons present by using the GLC conditions previously described (Sinden et al., 1980). In cases (see Table I) where the extracts contained more than one aglycon, or more than one glycoalkaloid pair, e.g., *S. brachycarpum* with solanidine (solanine–chaconine) and tomatidenol (α-solamarine–β-solamarine), the quantities of the individual aglycons found by measuring peak areas were used to determine the relative quantities of the different glycoalkaloid pairs. The quantity of each glycoalkaloid in the glycoalkaloid pair, e.g., solanine–chaconine with the common solanidine aglycon, was determined by apportioning the aglycon between the two glycoalkaloids by using peak area measurements from glycoalkaloid chromatograms.

For confirmation of the identities of the glycoalkaloids determined by the combined GLC analyses of glycoalkaloids and aglycons, ammoniacal precipitates were analyzed by using TLC methods (McCollum and Sinden, 1979). Relative quantities of the glycoalkaloids could also be estimated on TLC plates from the intensities of iodine staining, and these estimates were used to confirm the quantitative values obtained from the GLC chromatograms. Traces of unknown iodine staining compounds with R_f values similar to, or the same as, those for known glycoalkaloids were sometimes noted on TLC chromatograms. Also, traces of glycoalkaloids or other compounds with similar chromatographic characteristics were sometimes observed on GLC chromatograms. But unless an individual trace compound was present at a concentration of >3% of the total peak area on GLC chromatograms of glycoalkaloids or aglycons, it was not considered in the reported compositions of the leaf powders.

For confirmation of GLC identifications of the aglycons, the aglycon extracts were analyzed by the TLC methods described by Hunter et al. (1976), using one or more of the solvent systems suitable for aglycons from potato tissues. All TLC and GLC identifications of glycoalkaloids and their aglycons were made by comparing R_f values or retention times with those given by authentic standards in the different analytical systems. Tomatine and tomatidine were commercial standards (Sigma Chemical Co.). Other glycoalkaloids were obtained from plants known to contain the particular glycoalkaloid(s). Cochromatography with the standards was used in cases where the glycoalkaloid composition of the potato species had not previously been determined or the composition we found was not in agreement with that found by others. Saturation of the Δ^5 bond in aglycons, e.g., commersonine vs. dehydrocommersonine in *S. commersonii*, was determined by the method of Osman and Sinden (1977) since neither the normal GLC or TLC methods of analysis for glycoalkaloids or aglycons could reliably determine the difference between saturated and unsaturated glycoalkaloids.

RESULTS AND DISCUSSION

The foliar TGA and individual glycoalkaloid contents of the 16 *Solanum* species examined are shown in Table I. A wide range of TGA content (0.2–39.0 mg/g dry weight) was found. Most of the species contained higher TGA levels than the commercial cultivar, Katahdin. In *S. brachycarpum*, *S. chacoense*, and *S. hougasii*, the values were more than 10-fold higher.

Great diversity in individual glycoalkaloid composition was also found. Although most of the species contained only α-chaconine and α-solanine, several species contained substantial amounts of other glycoalkaloids not commonly associated with commercial cultivars. Most notable in this respect were *S. acaule* and *S. commersonii* which contained 100% demissine and 100% dehydrocommersonine, respectively, and *S. pinnatisectum* and *S. polyadenium*, both of which contained 100% tomatine. The water-soluble leptines I and II plus leptinines I and II were the predominant glycoalkaloids in the *S. chacoense* accession PI 320285 whereas α- and β-solamarines were the major glycoalkaloids in *S. brachycarpum*.

Extracts from accessions *S. acaule* PI 225620, *S. chacoense* PI 320285, and *S. polyadenium* PI 161728 apparently contained glycoalkaloids that did not precipitate completely when the extracts were subjected to the ammonia precipitation procedure employed. The presence of significant measurable concentrations of glycoalkaloids in ammoniacal supernatants from these extracts was indicated by the relatively large differences between the TGA concentrations obtained by titrating the ammoniacal precipitate and the concentrations obtained by first hydrolyzing the aqueous–acidic extract and then titrating the aglycon(s) isolated from the hydrolysate. Direct analyses of the glycoalkaloids remaining in the supernatants by TLC and GLC of aglycons liberated on hydrolysis showed that these relatively large quantities [2.8 mg/g dry weight for *S. acaule* to 15.0 mg/g dry weight for *S. chacoense* PI 320285] of glycoalkaloids were in fact present in these three supernatants. The high concentrations of glycoalkaloids in the ammoniacal supernatant of *S. chacoense* PI 320285 might be expected since some accessions of this species contain leptines (Kuhn and Löw, 1961; Sinden et al., 1980). Leptines are reported to be soluble in water and weak ammoniacal solutions (Kuhn and Löw, 1961). However, TLC analyses of the glycoalkaloids and GLC analyses of liberated aglycons from the ammoniacal supernatant fractions of *S. chacoense* PI 320285 showed that relatively large quantities (milligrams or more per gram dry weight) of α-solanine, α-chaconine and the leptinines (Kuhn and Löw, 1961; Sinden et al., 1980) were also present in the supernatants. Concentrations of demissine in *S. acaule* supernatants and tomatine in *S. polyadenium* supernatants were also greater than 1 mg/g dry weight of tissue. We cannot explain the presence of these relatively high concentrations of normally ammonia-precipitable glycoalkaloids, i.e., tomatine, demissine, leptinines, α-solanine, and α-chaconine, in ammoniacal supernatants from these foliar tissues. Perhaps leaves of these three accessions contain saponins or other compounds that may interfere with the precipitation of glycoalkaloids or their sedimentation by centrifugation. Nonnitrogenous sterols are known to occur in leaves of other *Solanum* spp., and such compounds can interfere with the precipitation process or the collection of the precipitated glycoalkaloids by centrifugation (Heftmann, 1967).

In three cases we were unable to conclusively determine the individual glycoalkaloid composition: (1) *S. berthaultii* apparently contains glycoalkaloids which have not been previously reported in tuber-bearing *Solanum* species. Solasodine was the major aglycon found in hydrolysates of *S. berthaultii*. The identity of solasodine was established by use of cochromatography (GLC; TLC) with the authentic compound, GLC–MS, NMR, melting point, and mixed melting point with tomatidenol. Compositions of the sugar moieties of the two major *S. berthaultii* glycoalkaloids were determined by GLC (Varma et al., 1973). The sugar moieties of these glycoalkaloids were two rhamnose and one glucose and one rhamnose, one glucose, and one galactose, respectively, corresponding to the trisaccharides of solamargine and solasonine. While the sugar moieties were not sequenced, the combined data on the aglycons, sugar moieties, and whole glycoalkaloids strongly suggest the presence of solamargine and solasonine in *S. berthaultii* foliage, although Schreiber (1968) reported the presence of only α-solanine and α-chaconine in leaves of

S. berthaultii. At present we cannot explain the differences between the latter report and our own. (2) *S. polyadenium* contained an unidentified compound which cochromatographed with tomatine on TLC but was not ammonia precipitable. Preliminary MS data indicate the absence of nitrogen, suggesting that the compound is not an alkaloid. (3) We could not identify any glycoalkaloids in *S. bulbocastanum* (WRF 1565) because of the extremely low TGA level. We suggest that foliage of this accession may be nearly glycoalkaloid free.

Most of our glycoalkaloid identifications agreed with the literature [see Schreiber (1968) for a review], but in addition to the data on *S. berthaultii* discussed above, there were some exceptions. *S. commersonii*, according to several reports, contains α-solanine, α-chaconine, and β-chaconine (Schreiber, 1955, 1963), whereas we found only one glycoalkaloid, dehydrocommersonine, in PI 320269. Dehydrocommersonine was first discovered in tissue culture roots of a *S. chacoense* accession by Zacharius and Osman (1977). In *S. brachycarpum* we found substantial amounts of α- and β-solamarine in addition to α-solanine and α-chaconine, whereas this species, under the synonym *S. verrucosum* (Schlechtd.), was reported to contain only α-solanine (Prokoshev et al., 1952). Several factors including different methods of analyses, environmental conditions, or intraspecific heterogeneity may be responsible for the differences between our data and those of others.

The fact that intraspecific heterogeneity in a wild species can alter individual glycoalkaloid composition has been documented previously (McCollum and Sinden, 1979) and is dramatically illustrated in Table I. The three accessions of *S. chacoense* we analyzed exhibited very different individual glycoalkaloid compositions. The predominant glycoalkaloids in the *S. chacoense* accession PI 320285 were leptinines I and II whereas none were found in PI 189220. The WRF 888 accession had only trace amounts of these ammonia-soluble glycoalkaloids.

Several of the species in Table I had not been previously analyzed for glycoalkaloid composition. These were *S. bulbocastanum* (discussed above), *S. fendleri*, *S. hjertingii*, *S. hougasii*, *S. medians*, *S. pinnatisectum*, and *S. polytrichon*. Accessions of *S. fendleri*, *S. medians*, and *S. polytrichon* contained α-solanine and α-chaconine at levels comparable to the levels found in *S. tuberosum* cv. Katahdin, while *S. hjertingii* and *S. hougasii* also contained α-solanine and α-chaconine but at levels 6–10 times higher than those in cv. Katahdin. *S. pinnatisectum* contained a moderately high level of tomatine but no other glycoalkaloids were detected.

Our data, in illustrating wide quantitative and qualitative variation in glycoalkaloid contents among wild, tuber-bearing *Solanum* species, emphasize the need for careful assessment of glycoalkaloids as a potato quality factor when such species are to be used in breeding programs. This could be particularly important in cases such as breeding for resistance to the potato leafhopper, *Empoasca fabae* (Harris), where glycoalkaloids can act as resistance factors (Tingey et al., 1978; Raman et al., 1979). However, none of the available methods of glycoalkaloid analysis provides rapid and simple estimates of both TGA levels and individual glycoalkaloid compositions in wild species because of the diversity of individual glycoalkaloids. Glycoalkaloids differ greatly in physical and chemical properties. For example, whereas the majority of glycoalkaloids can be precipitated by ammonia, some *Solanum* accessions (Kuhn and Löw, 1961; Table I) contain a sizable nonprecipitable fraction. In the absence of the appropriate methodology suitable for analysis of large breeding populations, we suggest analyses of selected plants using a combination of methods such as that described in this paper.

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Received for review December 15, 1980. Accepted June 15, 1981. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be available. This research was supported by the Science and Education Administration of the U.S. Department of Agriculture under Cooperative Research Grant No. 616-15-62 and the International Potato Center, Lima, Peru.